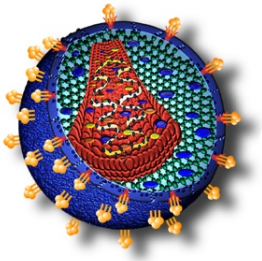


HIV Drug Resistance Program

National Cancer Institute at Frederick

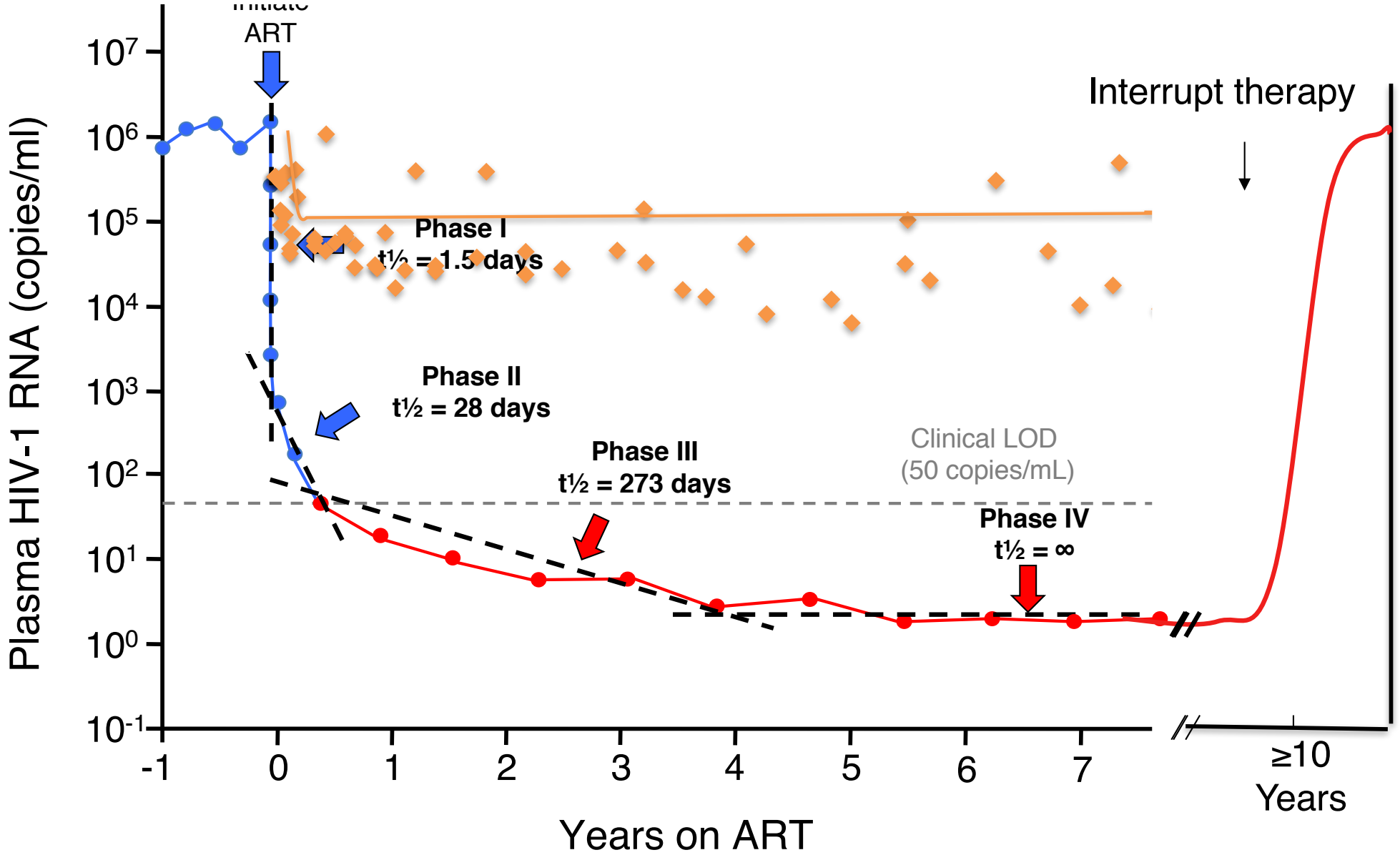
The Challenge of an HIV Cure:

Mechanism of HIV Persistence Despite Suppressive Antiretroviral Therapy

John M. Coffin

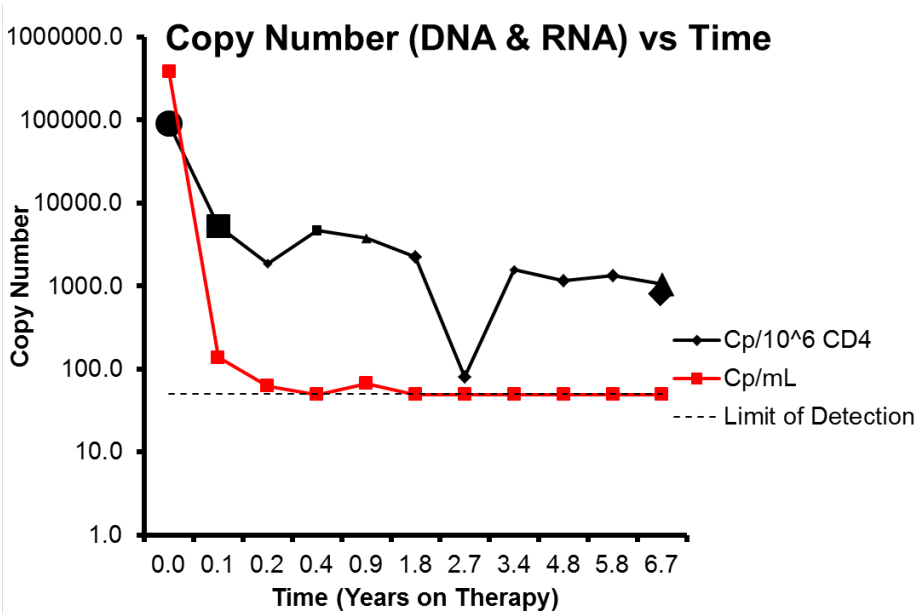
Tufts University

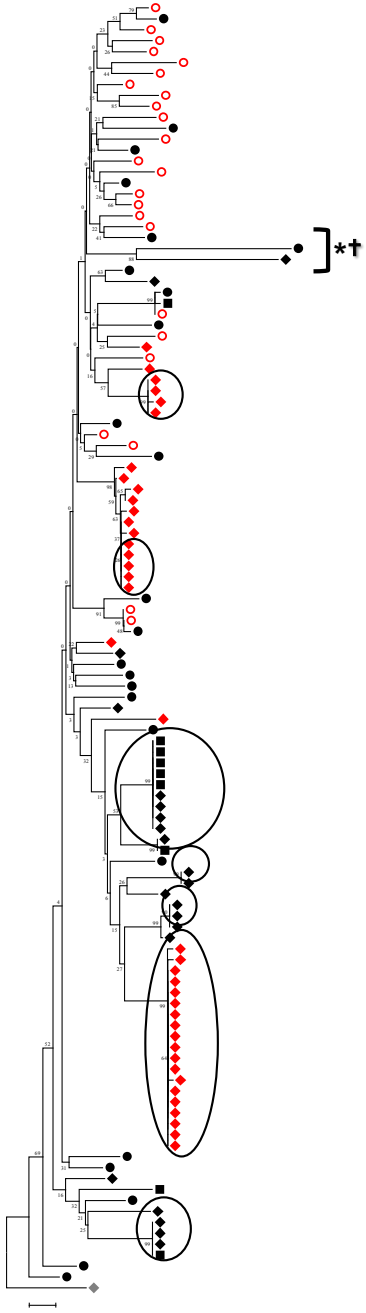
HIV-1 DNA Decays Much Less Than Plasma Viru RNA after Initiating ART



Courtesy Ben Hilldorfer, UPitt

Clonal Expansion of RNA and DNA Sequences (NO Evidence for Evolution)



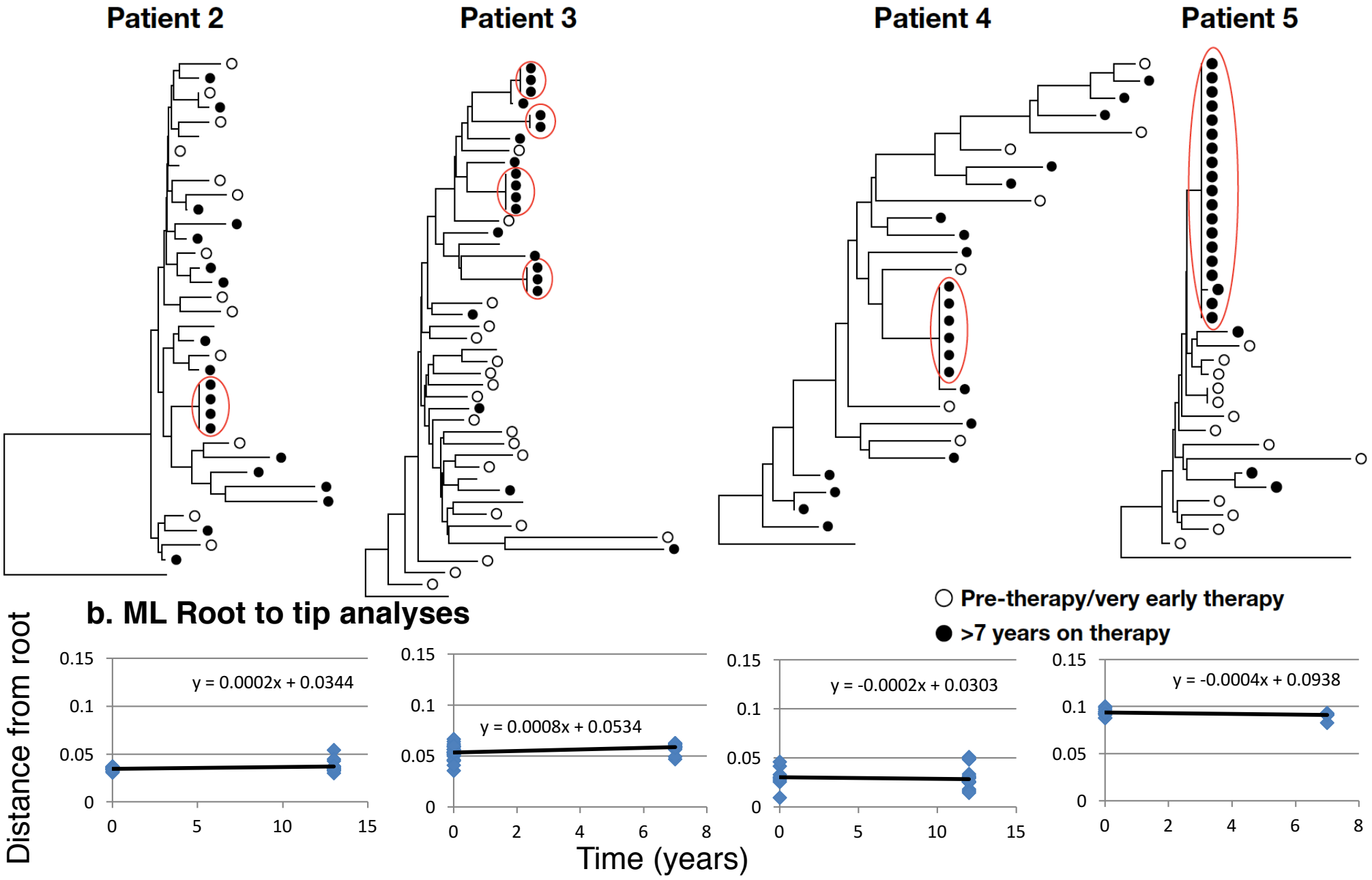


- 3 weeks
6 weeks
2.7 years
6.7 years
7.2 years
Reference
*G>A hypermutant
†stop codon

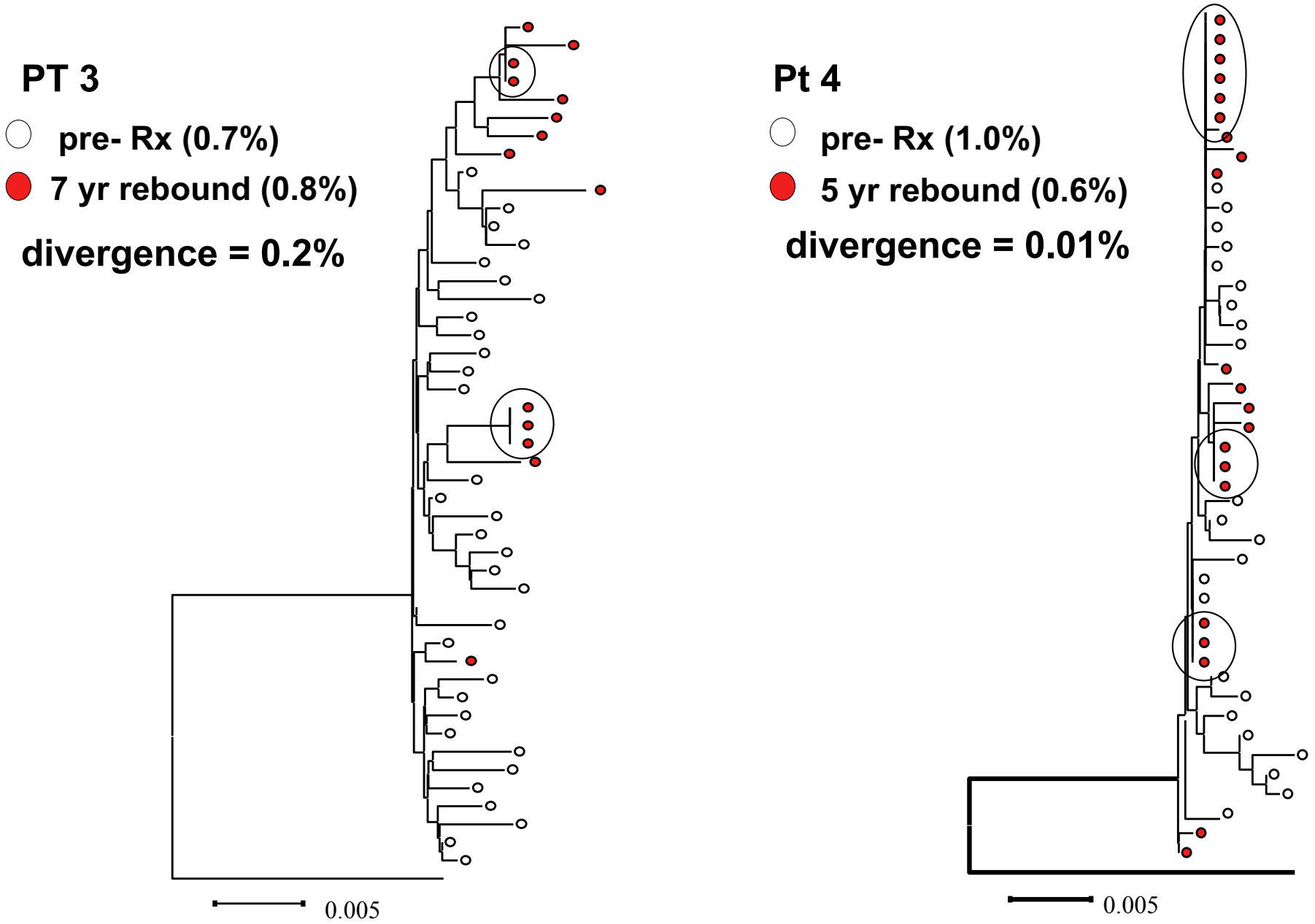
Different clonal populations of RNA and DNA appear after years of therapy

*Highly variable from patient to patient

No Detectable Evolution of HIV DNA in PBMCs after More than 7 Years of ART



No Evolution from Pre-therapy in Rebound Viremia After Long-term ART

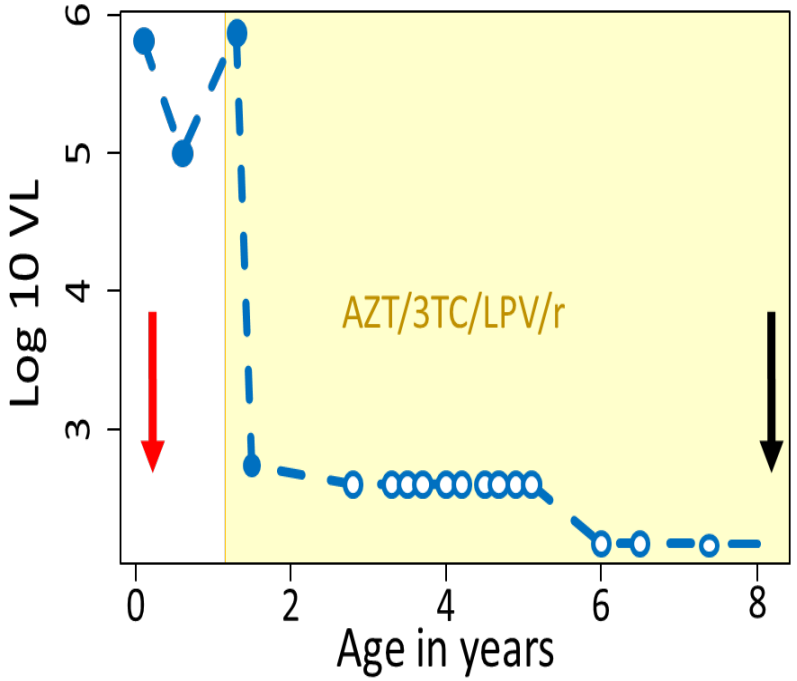


Obvious Viral Replication When Viremia is NOT Suppressed (Positive Control)

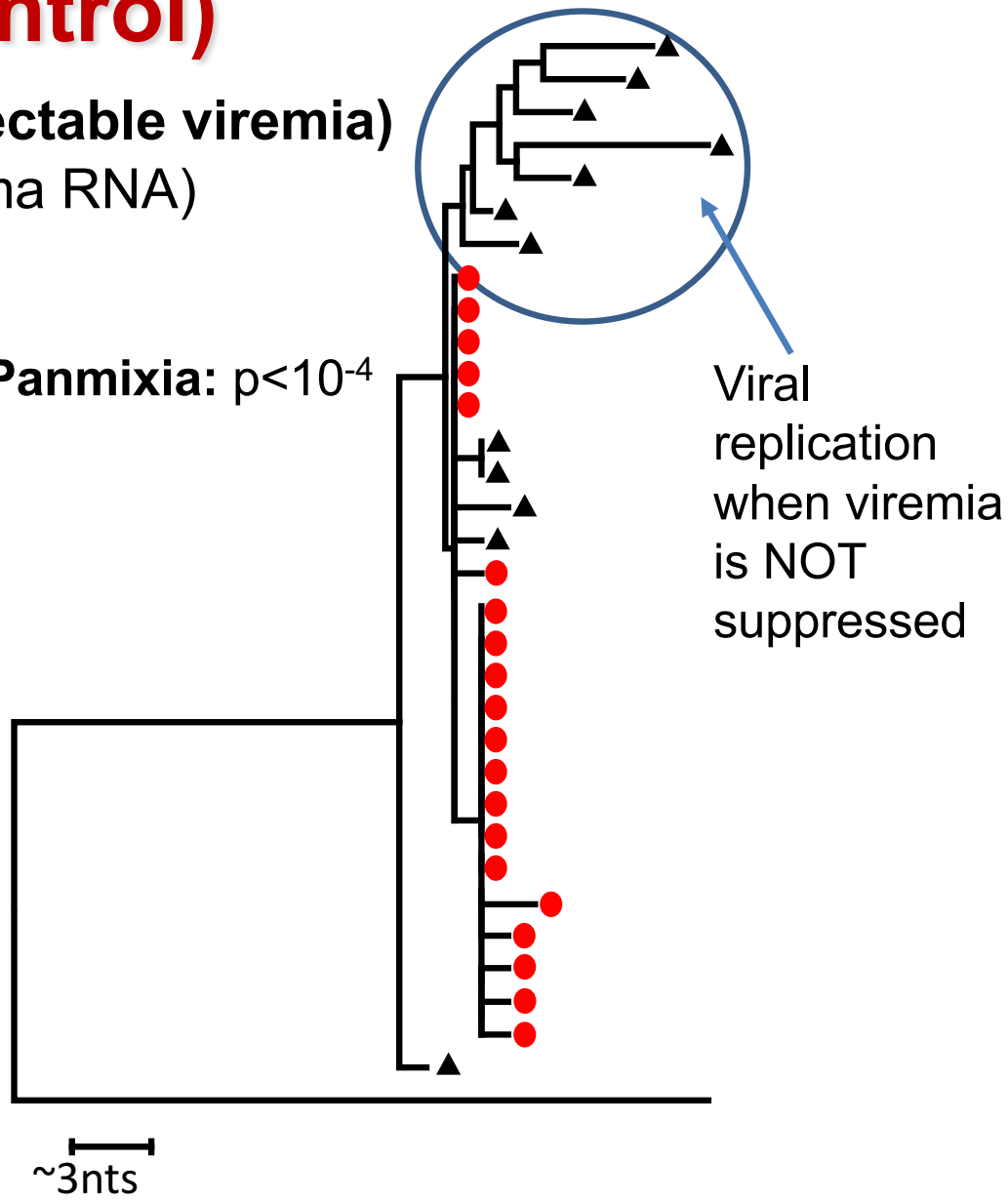
PID: 337286 (1.3 years of detectable viremia)

● 1.3 years before ART (Plasma RNA)

▲ 6.9 years on ART (DNA)



Panmixia: $p<10^{-4}$



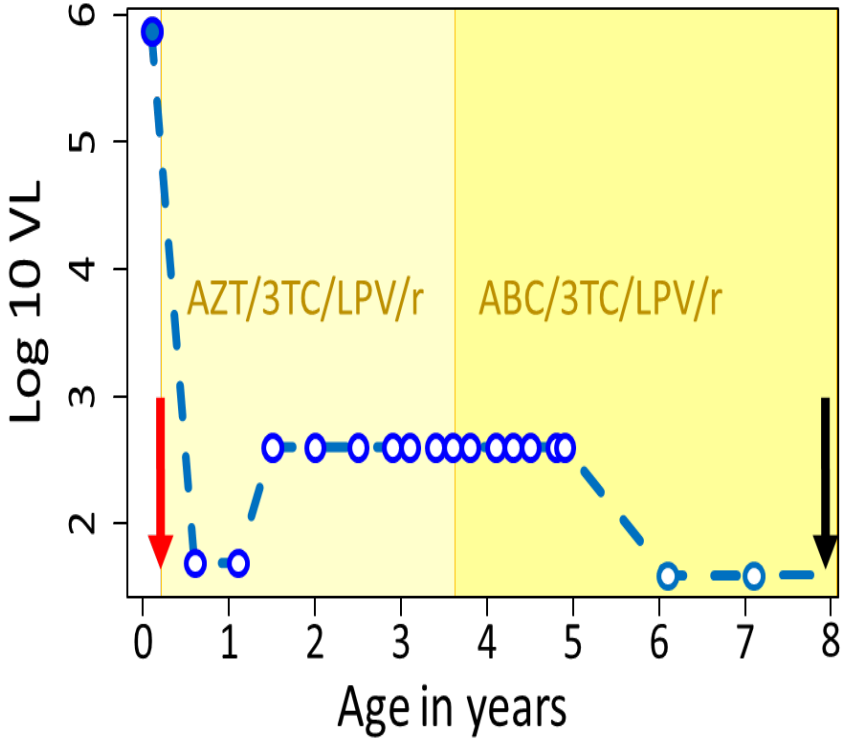
No Evidence of Viral Replication When Viremia is Suppressed

Initiated ART at 1.8 Months of Age

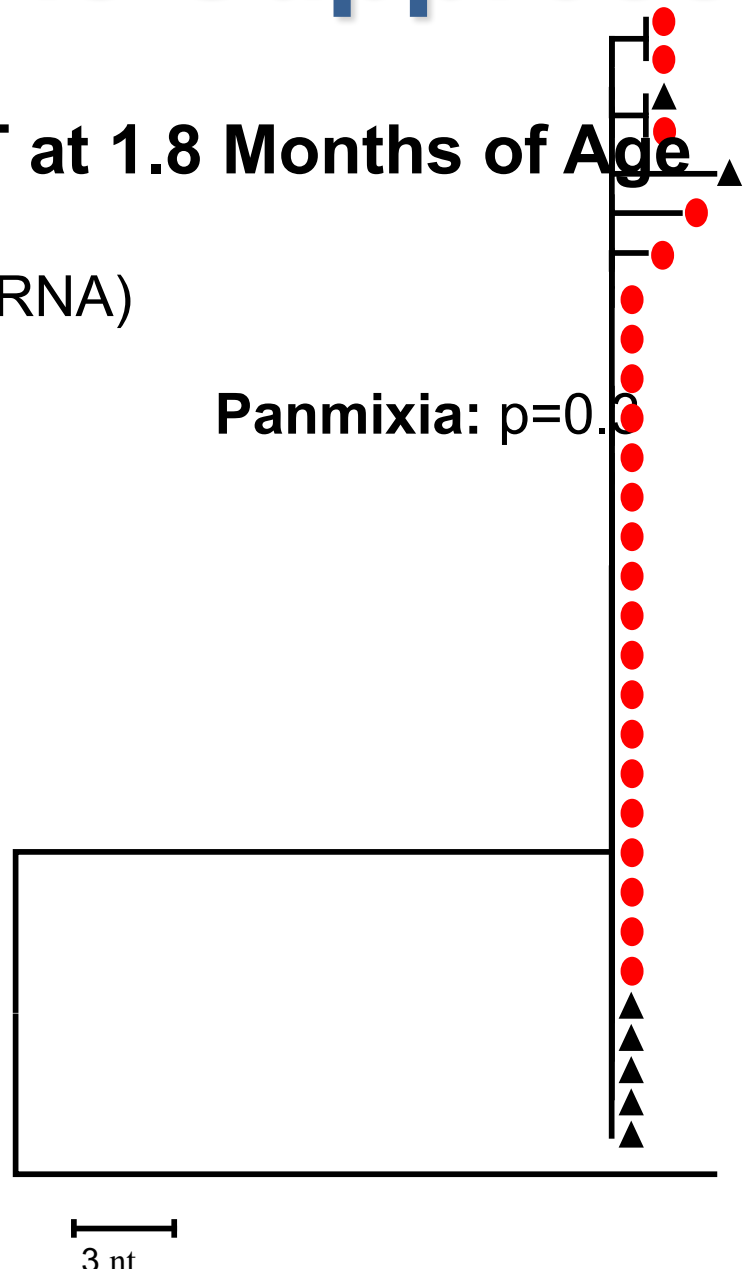
PID: 337916

● 14 days before ART (Plasma RNA)

▲ 8.1 years on ART (DNA)



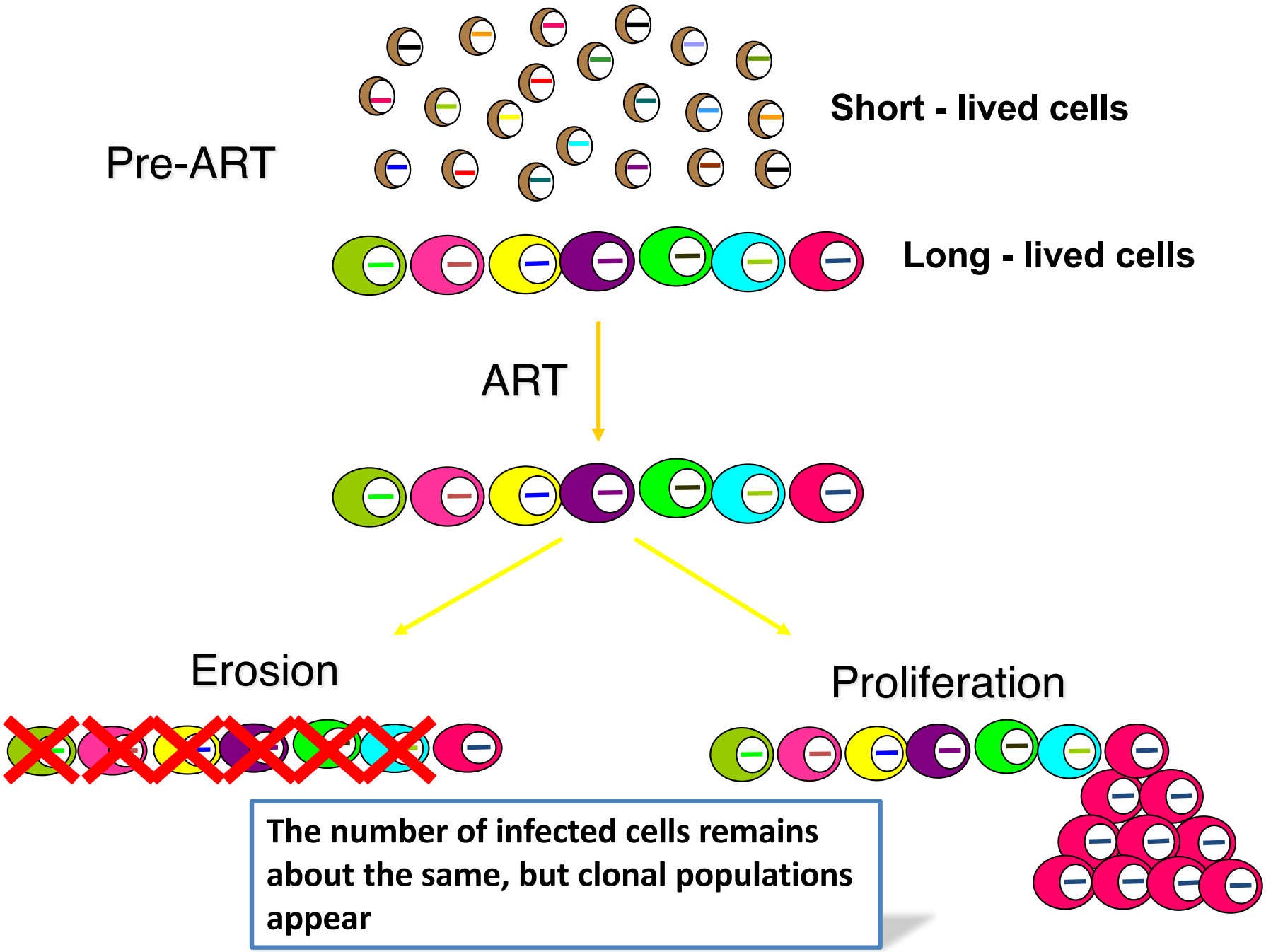
Panmixia: p=0.0



NO Evidence for a Role of HIV Replication in Maintaining the True Reservoir

What does maintain the reservoir?

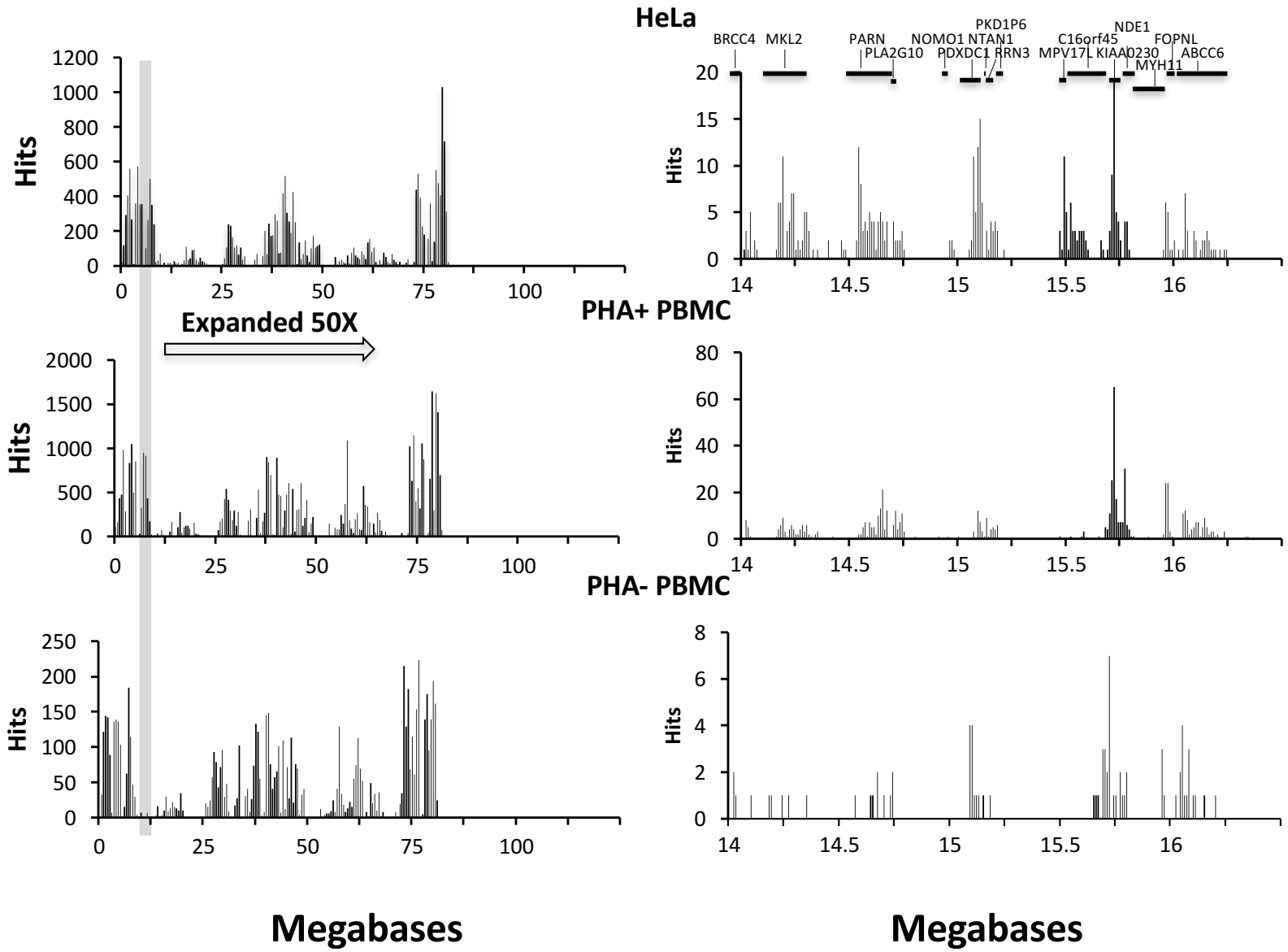
The Persistent Steady State



Integration Site Preferences 1.

- HIV DNA can integrate at many millions of possible sites in the cell gnome, and sites of integration can uniquely “tag” single infected cells and their progeny.
- In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e.., “hot spots”) and, selection after integration and by chance.
- We will look first at initial integration preferences in very different cells (PBMCs and HeLa cells) in culture.

Highly Similar Integration Site Preferences in HeLa and PBMC (Chromosome 16)



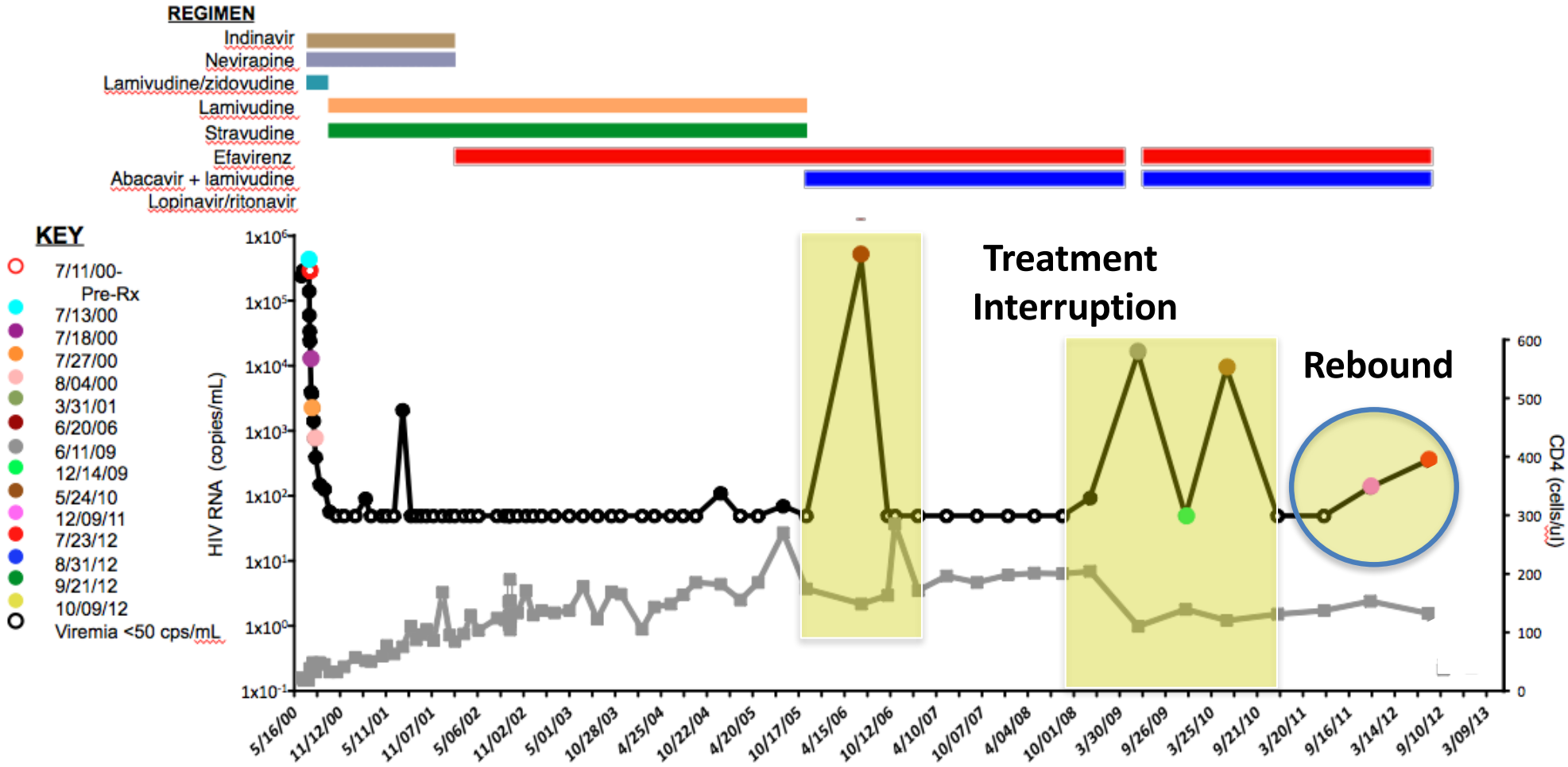
Integration Site Preferences 2. Selection for Specific Regions

In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e.., “hot spots”) and by selection after integration.

What happens to the distribution of integration sites in patients on long-term ART?

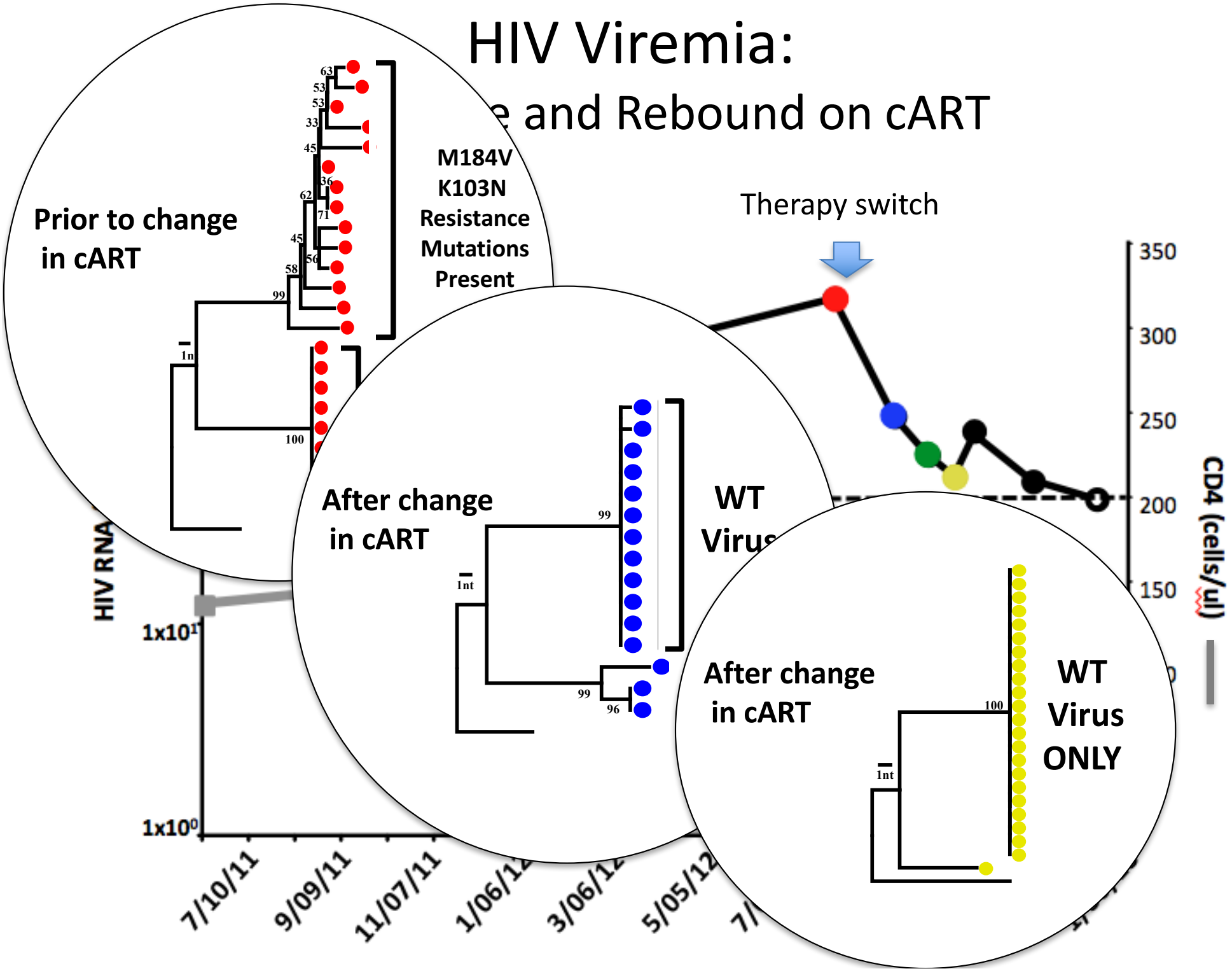
Case Report: Patient 1:

Persistence and Rebound of HIV Viremia on cART

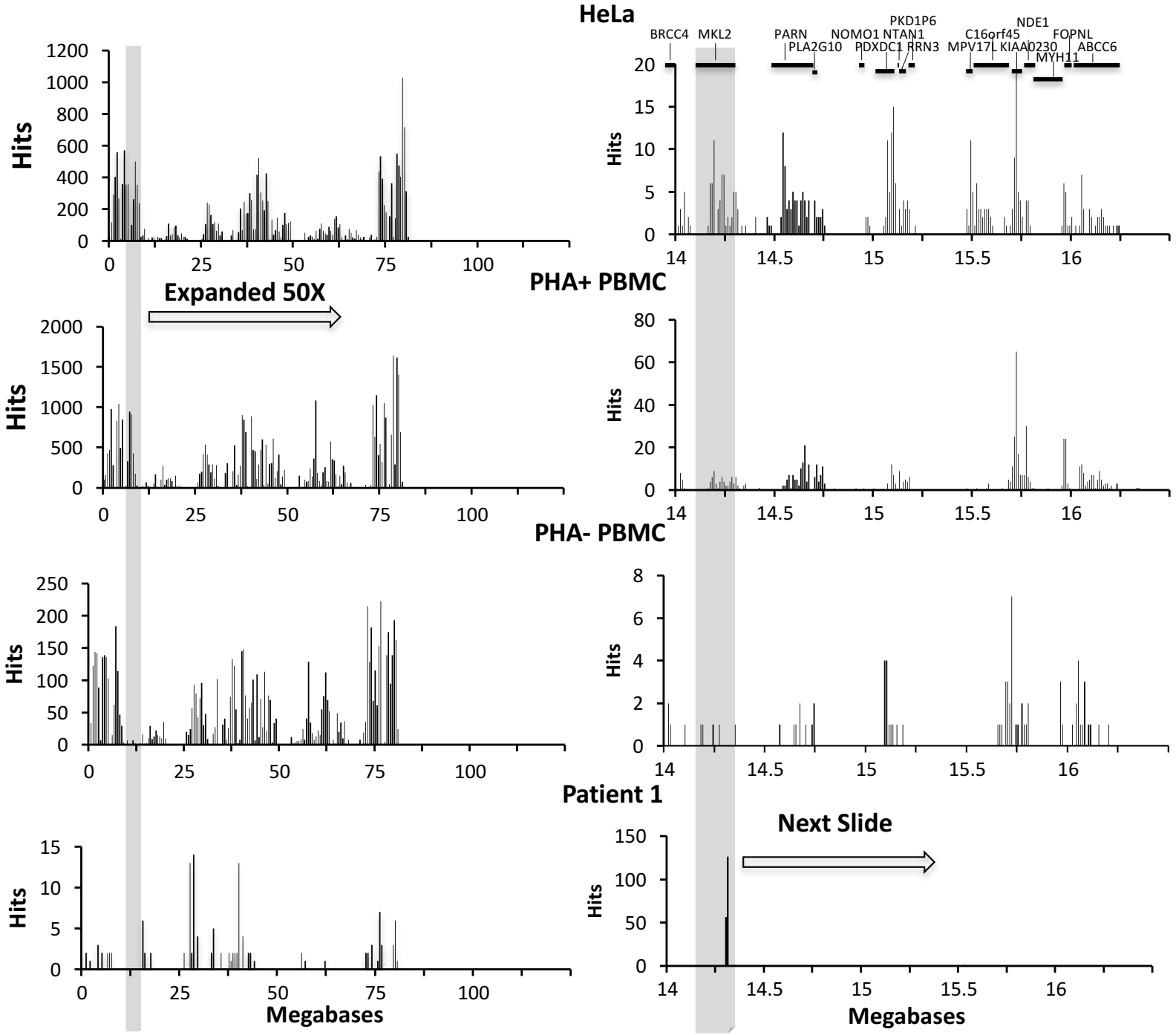


HIV Viremia:

e and Rebound on cART

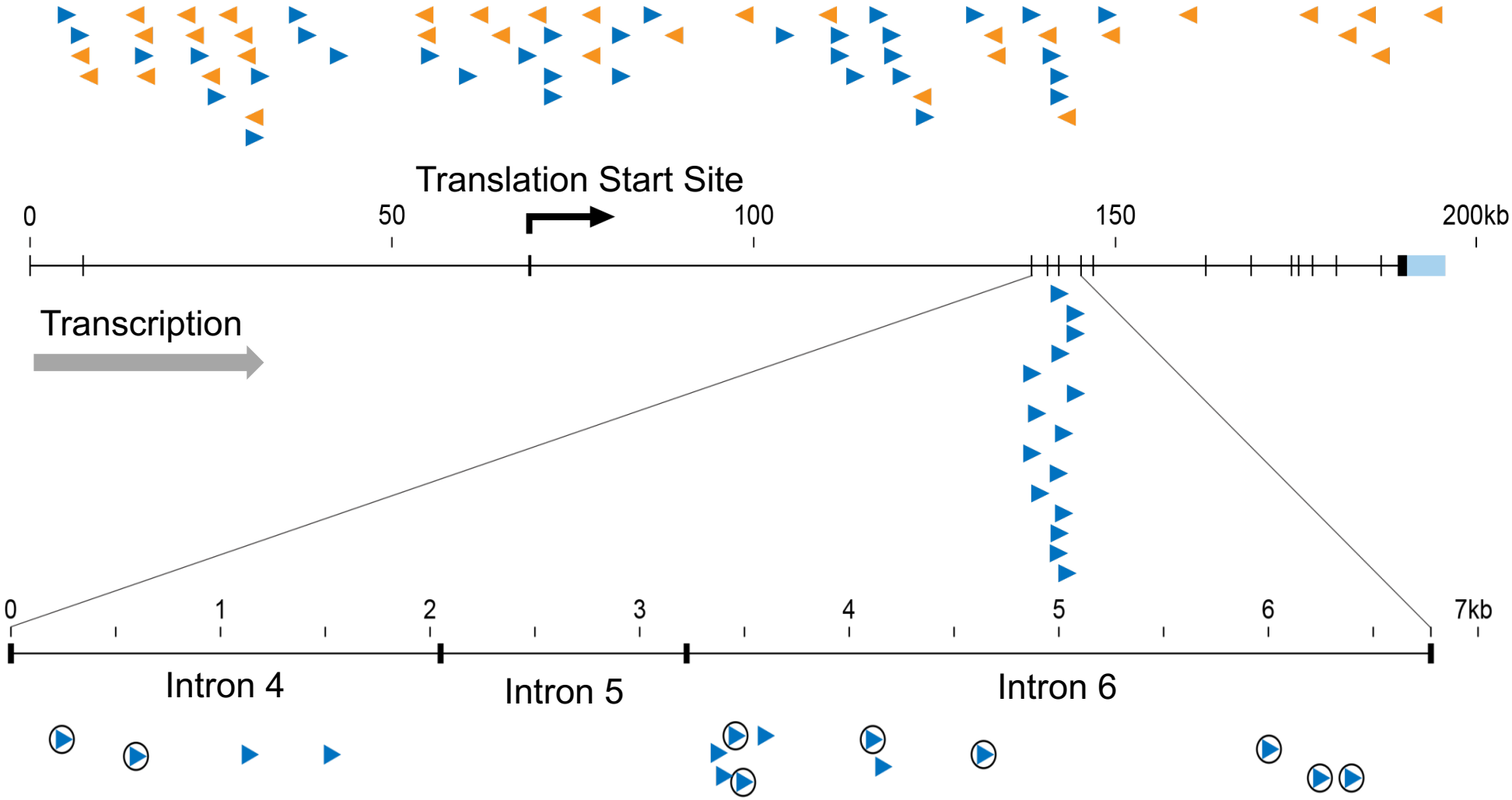


Integration Sites in Patient 1 Chr 16

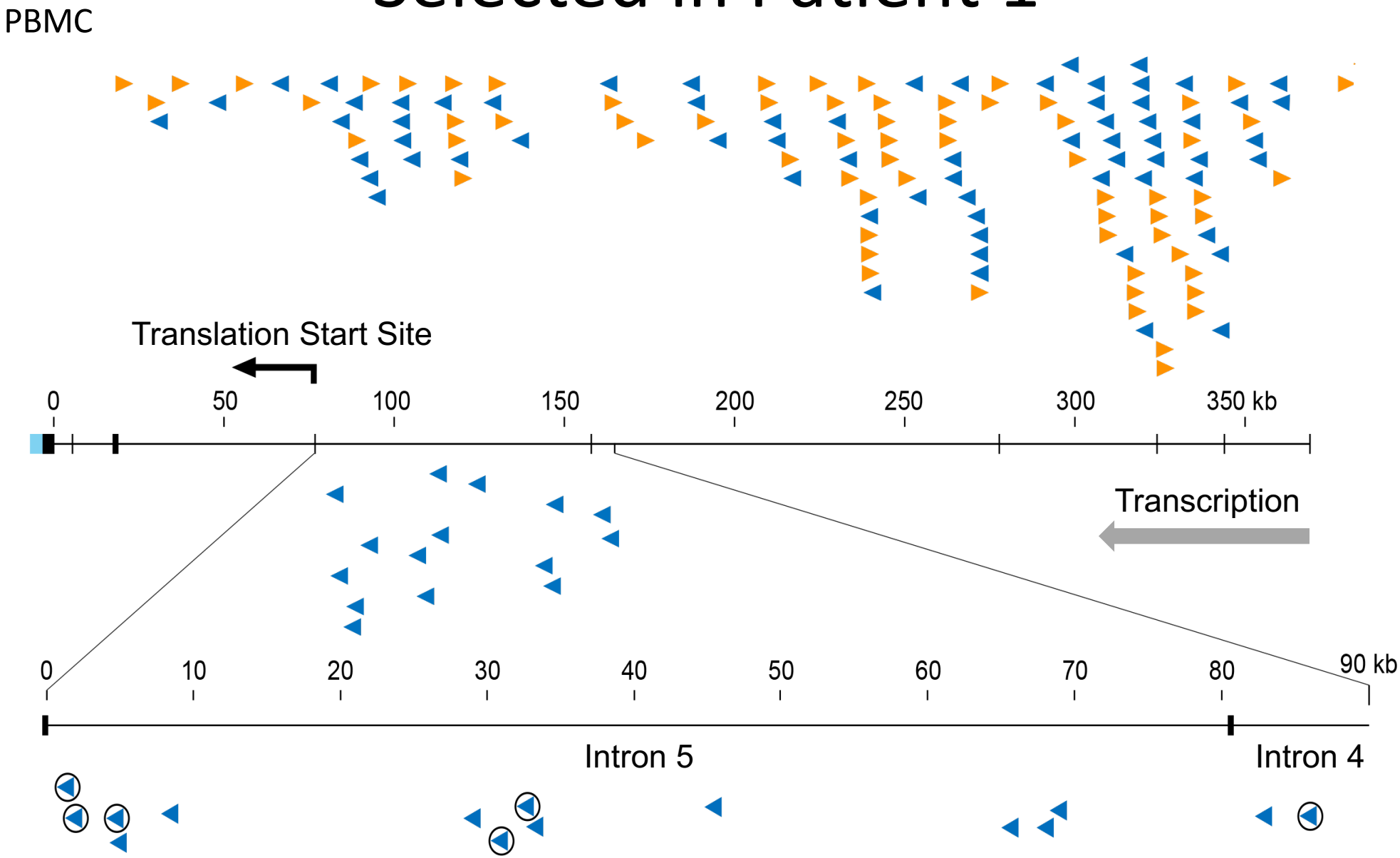


Integration Sites in MKL2 in Patient 1

PBMC



Integrations in BACH2 Are also Selected in Patient 1



A Popular Hypothesis

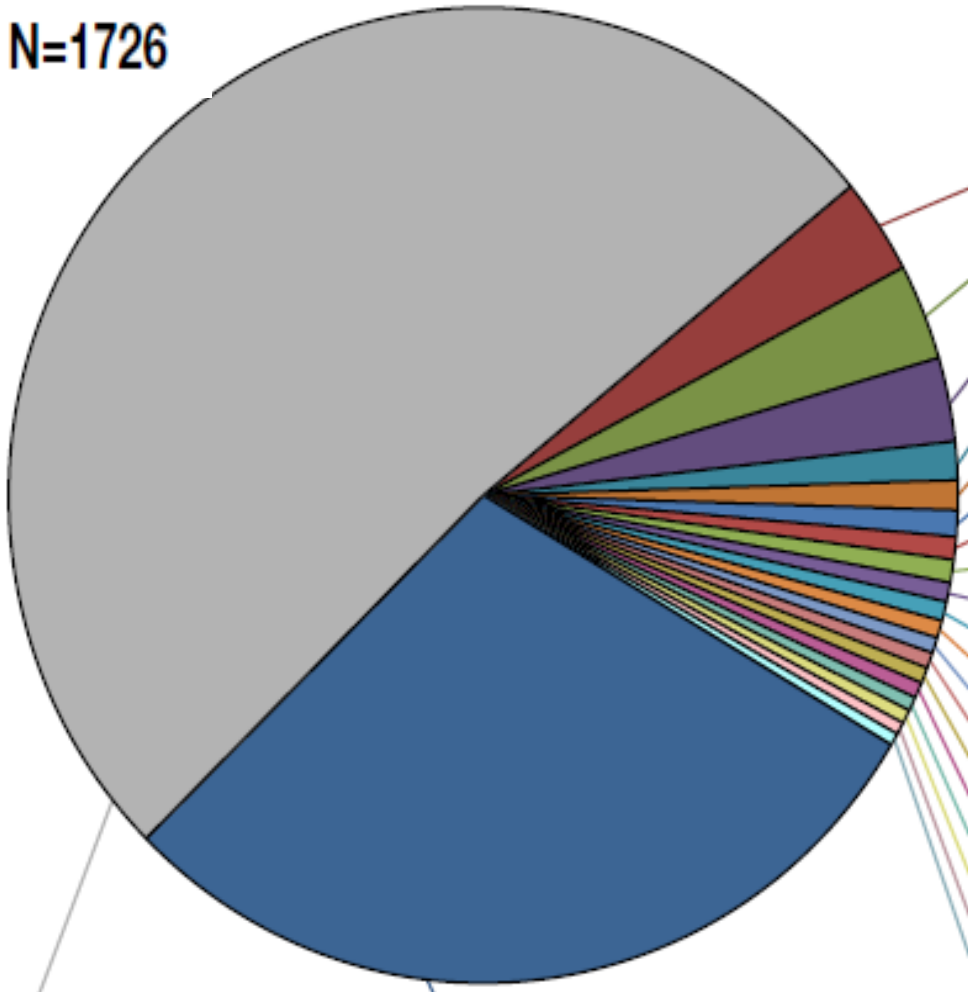
- In addition to clonally expended cells, much of the “reservoir” is in unexpanded, resting CdD4 cells.
- Activation of such cells to divide by antigen, homeostatic, or other signals will also activate expression and production from cells with nondefective (infectious) proviruses.
- Such cells will die due to virus replication or the immune response.

Therefore:

Clonally expanded HIV infected CD4+ T cells will only harbor defective proviruses.

FALSIFIED

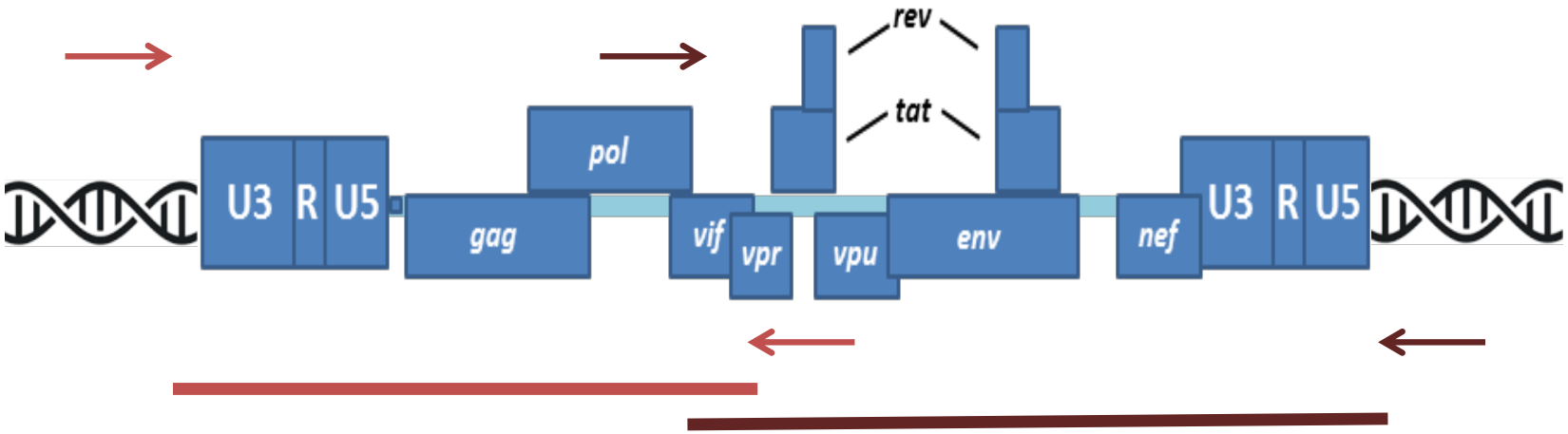
Integration Site Analysis Identifies Highly Expanded Clones in Patient 1



Gene	# of integrants	% of total integrants
Ambiguous	55	3.2
Unknown	55	3.2
MKL2a	48	2.8
MKL2b	22	1.3
ATP6V1G3	17	1.0
Unknown	15	0.9
TNFSF13B	13	0.8
Unknown	13	0.8
DDX6	11	0.6
MKL2	11	0.6
FSIP1	10	0.6
KIAA0319L	9	0.5
MKL2c	9	0.5
MKL2d	9	0.5
Unknown	9	0.5
PABPC1L	8	0.5
RBM15B	8	0.5
CEPT1	7	0.4
STAT5B	7	0.4

Maldarelli et al. Science 2014

AMBI-1 Full Provirus is Intact



Amplify in overlapping fragments and sequence

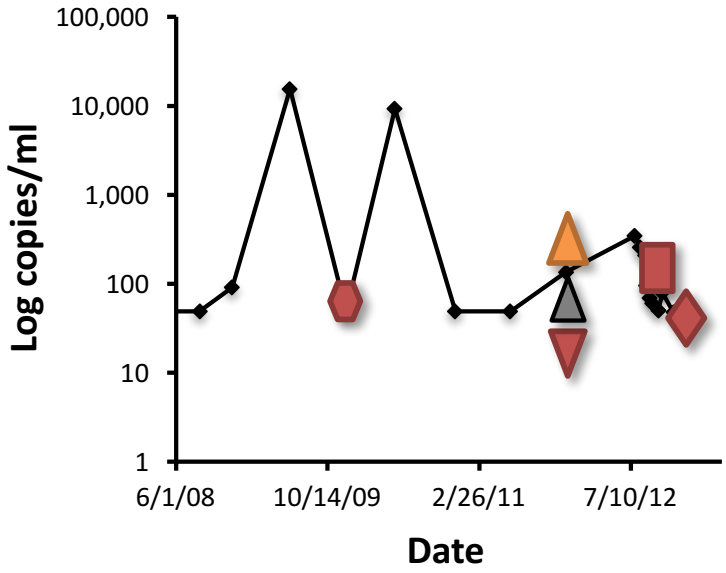
Full length genome:

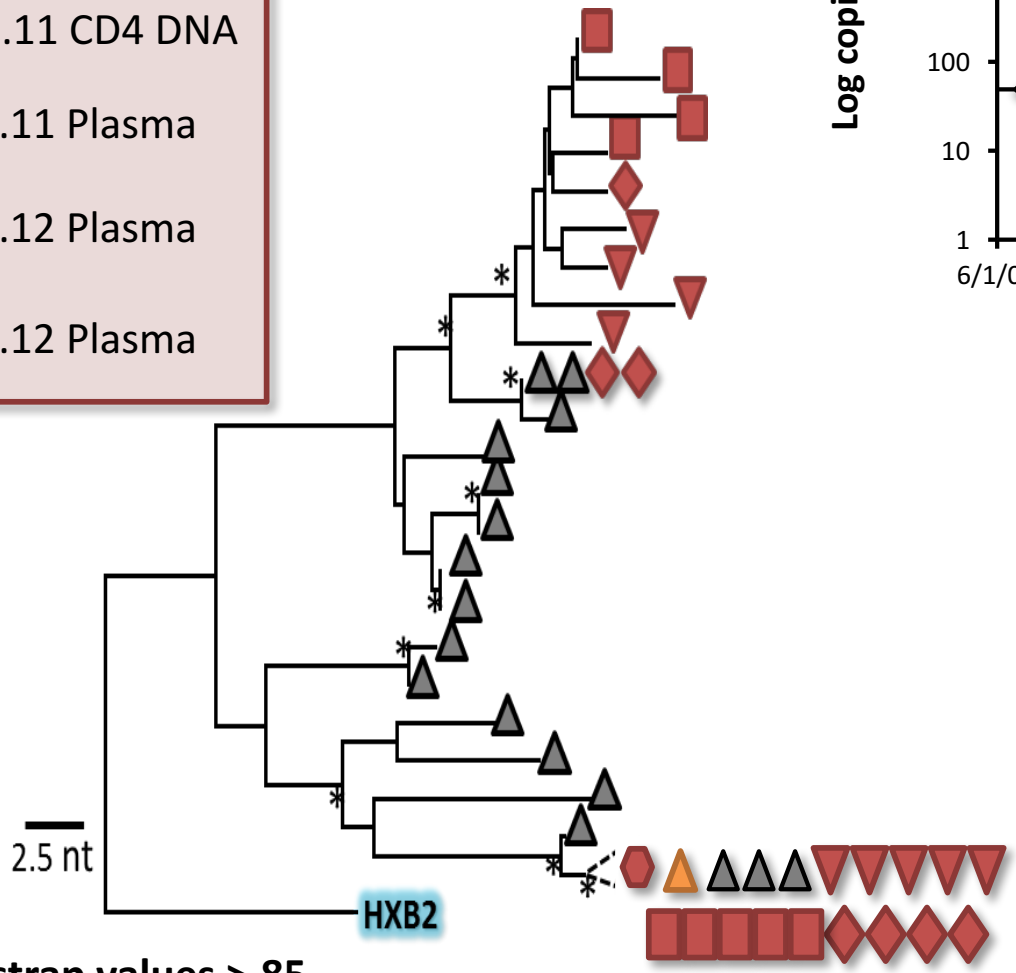
- No deletions
- No frame shifts
- No stop codons
- No Drug Resistance Mutations
- Predicted CCR5 tropic *env*
- No new CTL escape mutations

The Predominant Virus in Plasma is Produced by the AMBI-1 Provirus

P6-RT Sequences

- AMBI-1 provirus
- HIV RNA 12.14.09 Plasma
- HIV DNA 12.09.11 CD4 DNA
- HIV RNA 12.09.11 Plasma
- HIV RNA 07.23.12 Plasma
- HIV RNA 08.31.12 Plasma

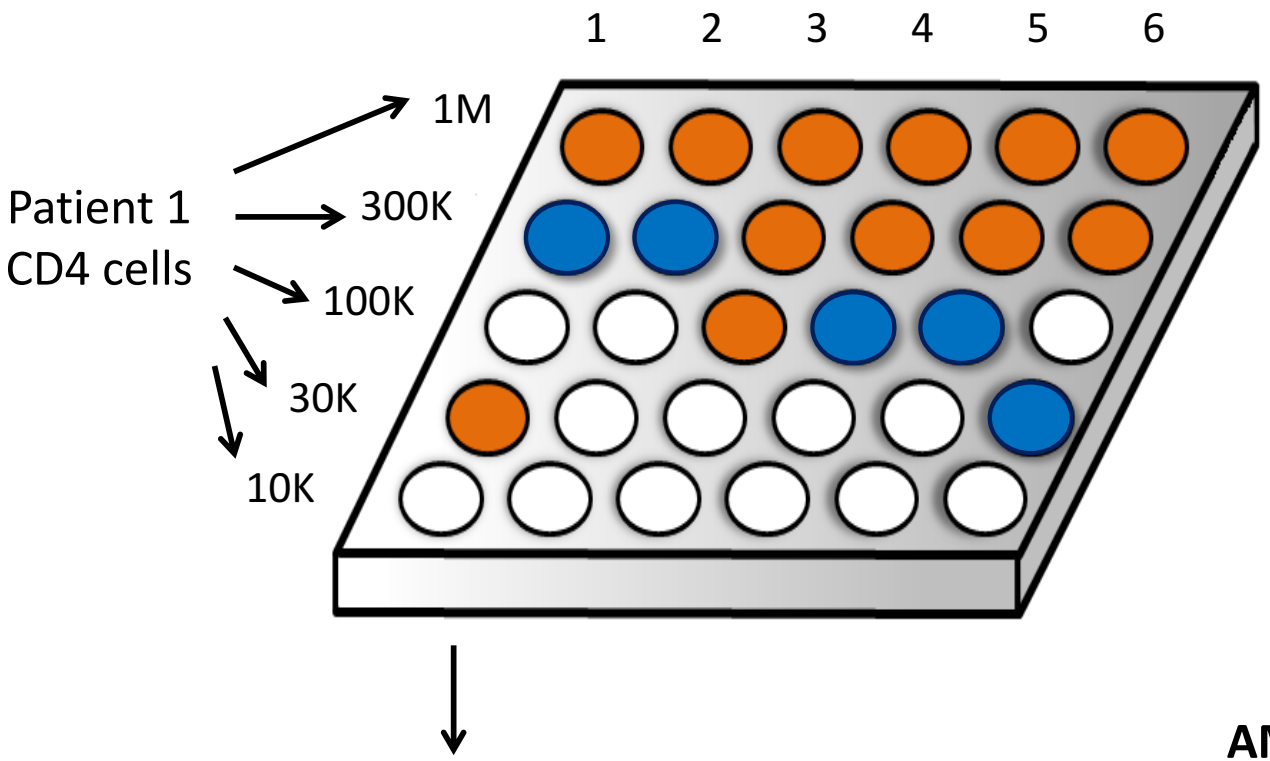




Predominant Plasma Clone (PPC)

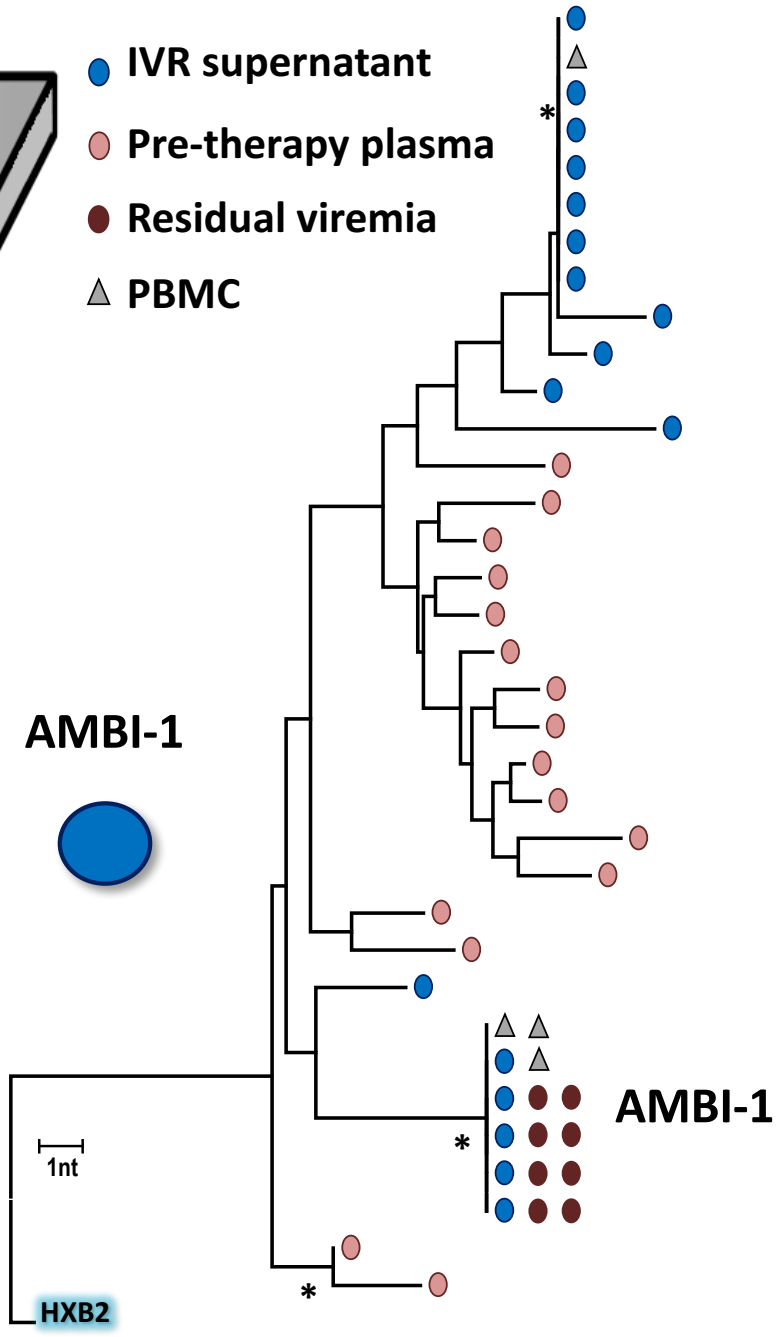
***Bootstrap values >.85**

Identification of Infectious Variants Recovered from CD4s



HIV from p24 positive wells was sequenced and aligned with patient plasma and proviral sequences

Sequences of multiple variants recovered in vitro are IDENTICAL to AMBI-1



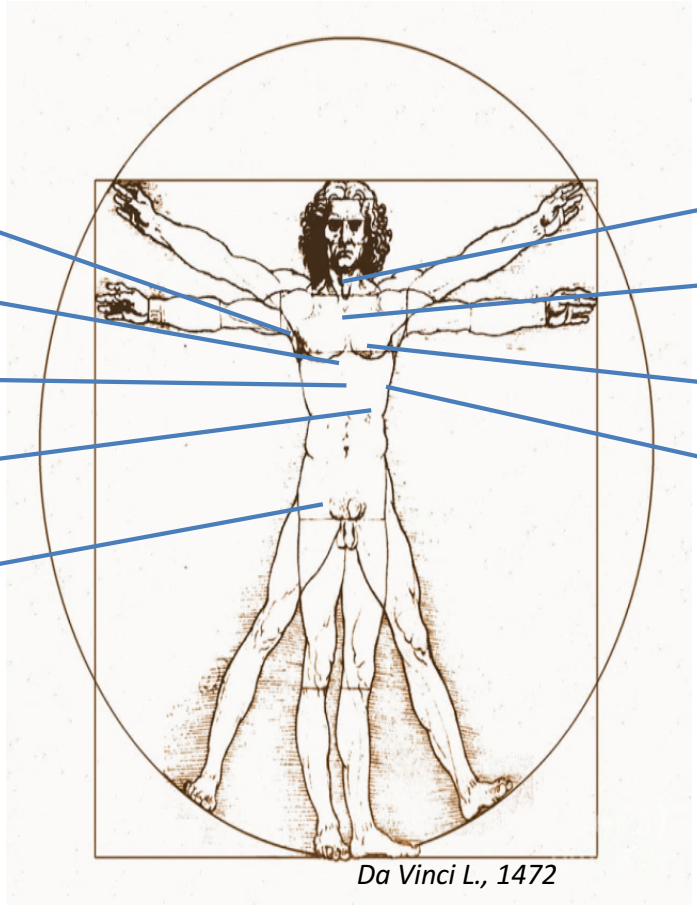
A Highly Expanded HIV Clone Carries an Infectious Provirus

This is the only known case where we can identify and characterize a clone of latently-infected cells responsible for infectious virus in blood

What is the epigenetic state of this provirus?
(We don't know yet)

What is driving clonal expansion?
(We know a little bit)

Cells with AMBI-1 Provirus are Widely Distributed and Enriched in Tumor Tissue



- Axillary LN
- Peri-hilar LN
- Para-aortic LN
- Spleen
- Inguinal LN
- Larynx Tumor
- Tracheal Tumor
- Intra-cardiac Tumor
- Chest mass Tumor
- PBMC
- Plasma

Total single genome sequences: 317

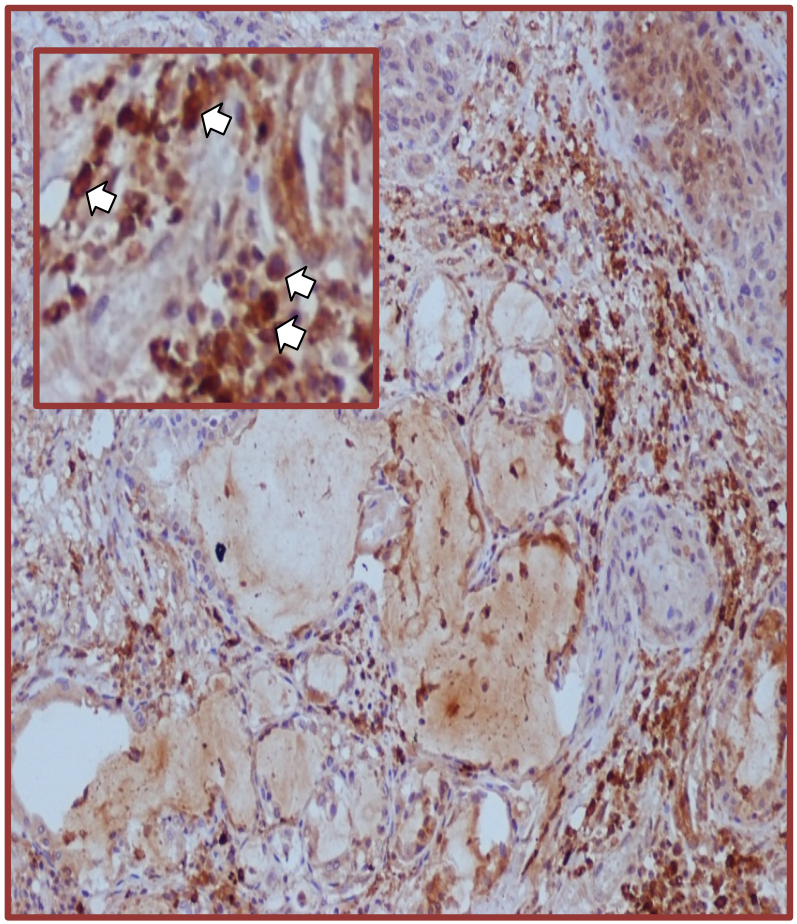
AMBI-1 present

Thanks to Francesco Simonetti

Cells with AMBI-1 Provirus are Widely Distributed and Enriched in Tumor Tissue

CD4+ IHC on metastatic tissue

H+E on metastatic tissue



P63+ HPV- EBV-

Number of HIV-DNA sequences in Different Tissues

p=0.0002	Lymphoid tissues	Metastatic lesions
AMBI-1	6	21
Other clonal populations	90	65
Non-clonal	57	36
Total Sequences	153	122

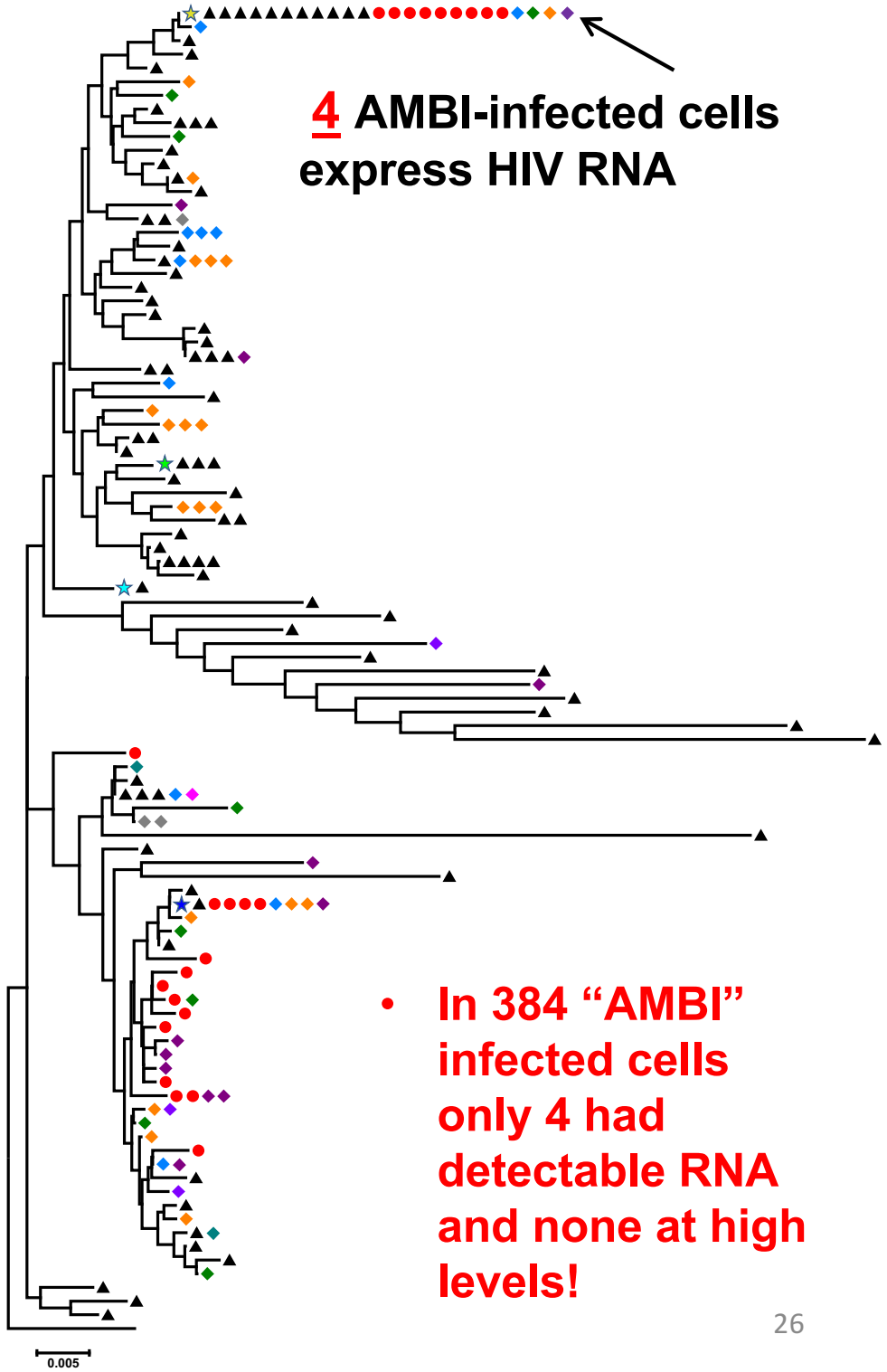
Thanks to Francesco Simonetti

Small Fraction of Cells from within an Expanded Clone Express HIV RNA

HIV DNA

Plasma HIV RNA

8 Individual HIV CAR extractions



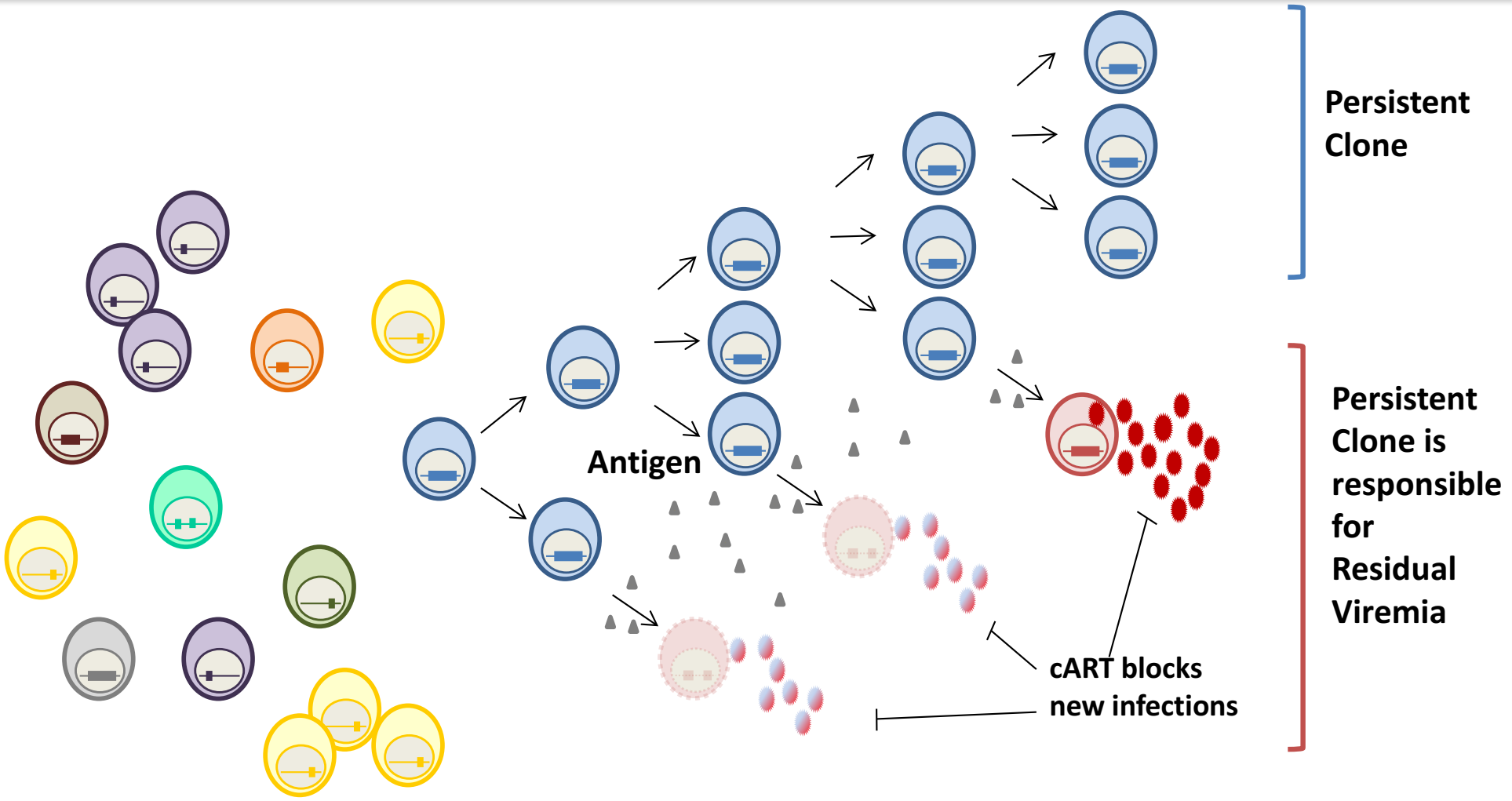
- In 384 "AMBI" infected cells only 4 had detectable RNA and none at high levels!

Cells infected with an intact provirus can expand and produce infectious HIV

Despite the potential for viral cytopathic effect and immune-mediated killing of expressing cells, cell clones can both expand and harbor intact proviruses that produce infectious virus over time.

Ca 1% of cells with the AMBI-1 provirus express small amounts of RNA at any one time.

(M. Kearney)



Summary

- Chromosomal integration patterns are very similar among very different cell types.
- Integration sites are readily identified in substantial numbers in cells from HIV infected individuals
- Integration site may influence persistence and clonal expansion and therefore, the virus that rebounds
- Clonal variants in plasma can be mapped to individual integration sites
- HIV infected cells can undergo localization
- Expanded clones CAN contain infectious proviruses
- AMBI-1 clone expanded in response to tumor antigen??

Acknowledgments

- HIV Drug Resistance Program
  - Stephen Hughes
    - Andrea Ferris
- Leidos Biomedical Inc
  - Xiaolin Wu
    - Ling Su
    - David Wells
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    - Jeff Lifson
- University of Pittsburgh
  - John Mellors
    - Michelle Sobolewski
    - John Bui
- Stellenbosch University
  - -- Gert Van Zyl
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    - Jennifer Bell
    - Junko Hattori
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  - Bob Yarchoan
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- Translational Research Unit
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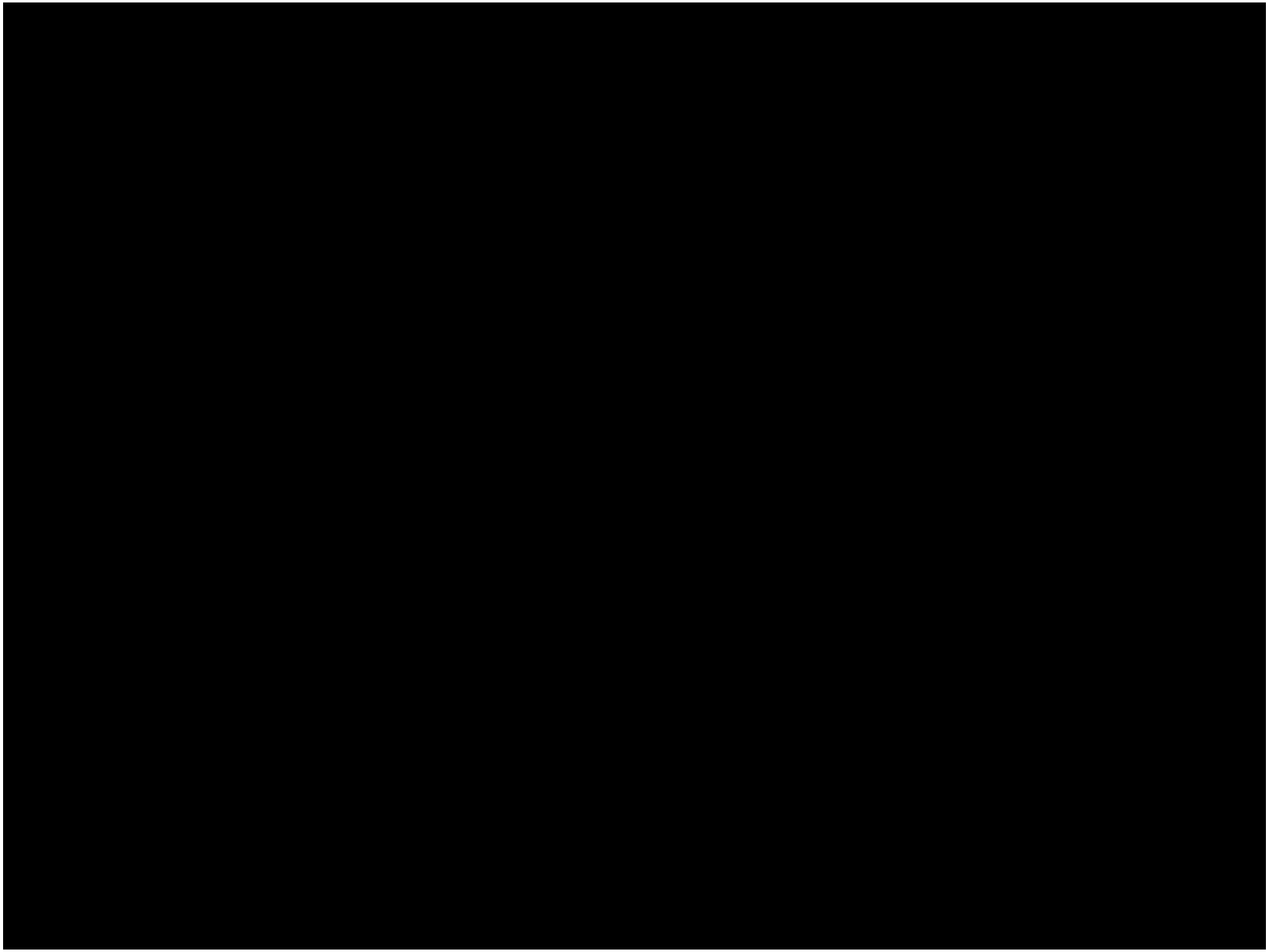
    - Ann Wiegand
    - Valerie Boltz
    - Jon Spindler
    - Wei Shao
    - Andrew Musick

NIH Bench to Bedside Program

NCI Contract No. HHSN261200800001E

And the Patient Participants!



What about intracellular RNA expression on and off ART?

Similar Diversities were Found in DNA, RNA, and Plasma in am Untreated Patient

Viral Load: 206,800 (iSCA)

CAR APD = 1.2%

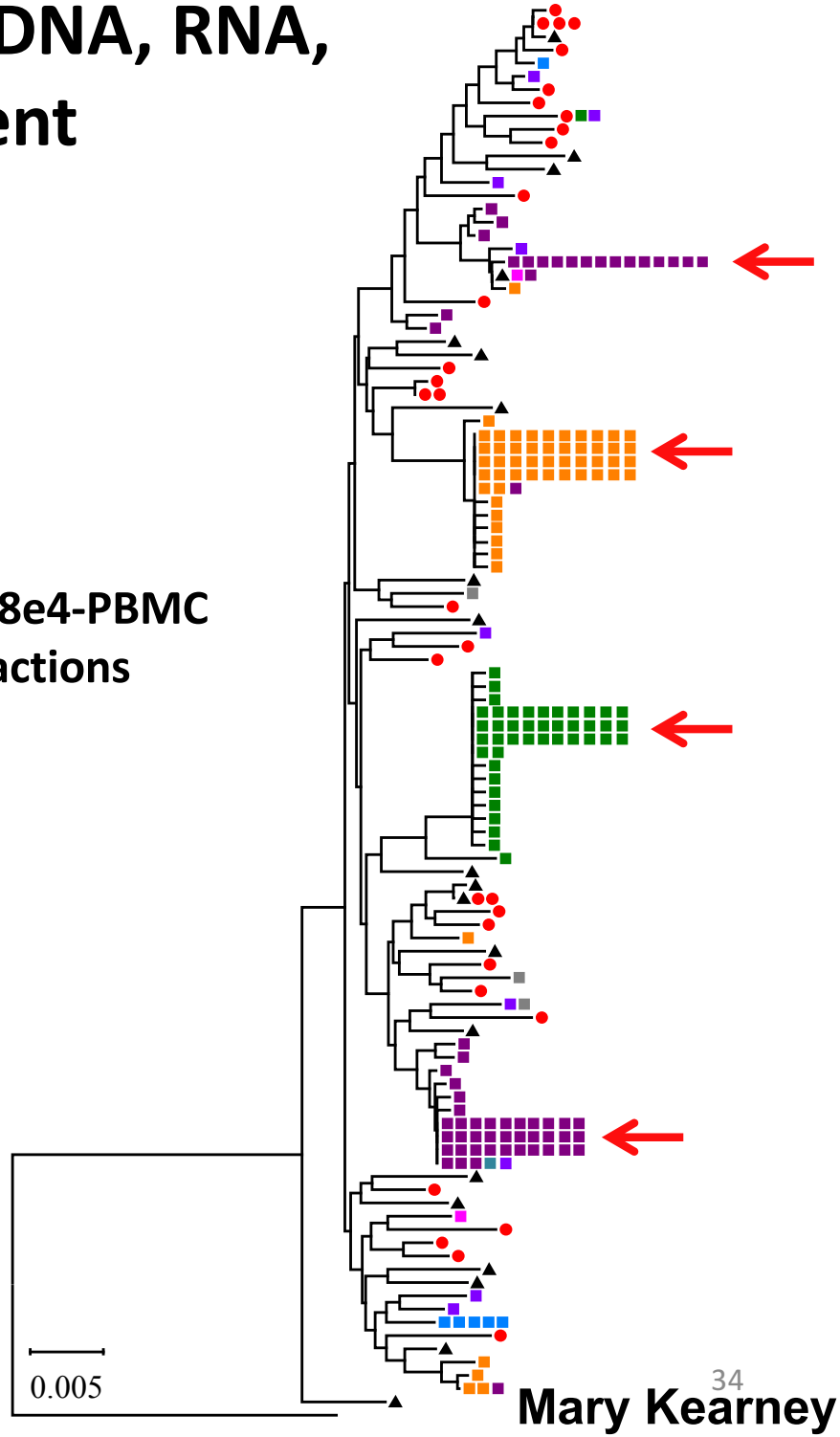
CAD APD = 1.7%

Plasma APD = 1.6%

HIV-1 DNA

Plasma RNA

Individual 3.18e4-PBMC CAR extractions



Cells Expressing RNA = 34

% of Infected PBMCs Expressing RNA = 10%

% Hyper-producing Cells = 12%

Mary Kearney

Similar Diversities were Found in DNA, RNA, and Plasma – Treated Patient

Viral Load: <0.6 (iSCA)

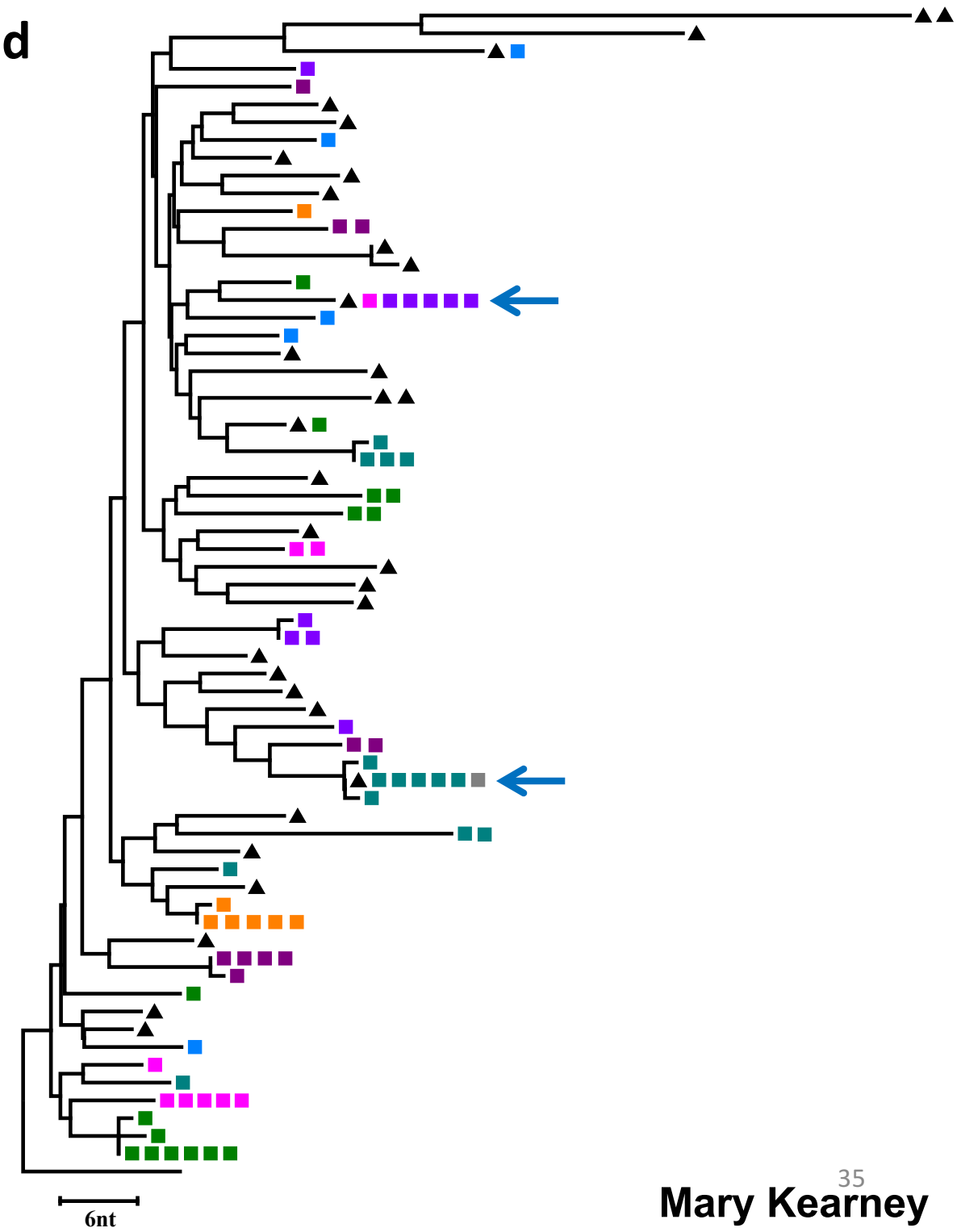
CAR APD = 2.0%

CAD APD = 2.8%

▲ HIV-1 DNA

Individual 1.07e5-PBMC CAR extractions

← Matches across compartments



Cells Expressing RNA = 31

% of Infected PBMCs Expressing RNA = 7%

% Hyper-producing Cells = 0%

Mary Kearney

No Difference in Cells Expressing HIV RNA

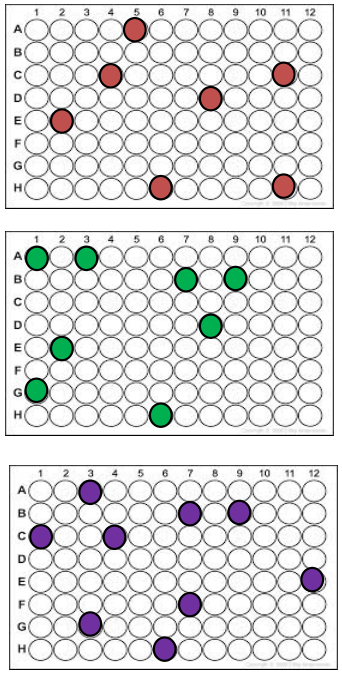
	PID	CD4 %	RNA c/ml iSCA	iCAD	iCAR	fPVE (%)	CARD-SGS(%)	expressing cells (%)
untreated	PID83	22.0%	17340	743	850	8	4	6%
	PID71	27.8%	206800	1567	2181	4	10	7%
	PID82	44.4%	75	55	76	13	33	23%
	PID69	29.9%	1533	79	27	6	6	6%
	PID97	24.9%	3660	1092	172	3	15	9%
								Median= 7%
treated	PID84	31.4%	1.9	103	12	4	6	5%
	PID90		<0.6	874	117	11	4	8%
	PID45	24.0%	<0.6	677	229	3	9	6%
	PID35	51.4%	<0.6	548	157	4	7	6%
	PID05	25.9%	<0.6	168	85	3	12	8%
								Median= 6%

Intracellular RNA and DNA Expression Assay (CARD)

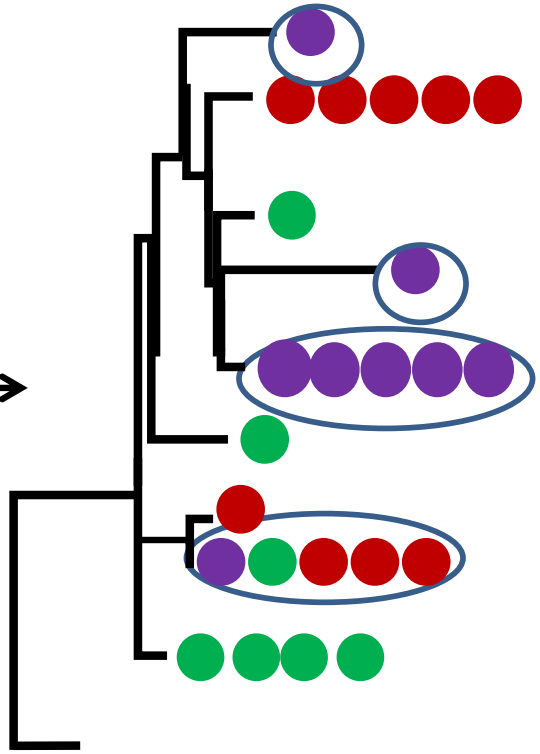
1) Collect PBMCs

2) Aliquot to ~2-10 HIV expressing cells per vial

3) Sequence single HIV CAR molecules



4) Analyze phylogenetically



- *Different RNA sequences in an aliquot are from expression from different cells*
- *Identical RNA sequences in an aliquot are higher levels of expression from the same cell*
- *Identical RNA sequences from different aliquots are from clonal siblings when collected during ART*

Mary Kearney`